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**BACTERICIDAL EFFICACY OF A PERSONAL
WATER PURIFICATION STRAW**

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14. ABSTRACT The straw was designed to provide easy to carry, portable water purification to soldiers in the field. The purification process takes place in a single pass while drinking water through the straw. The purification system incorporates a multistage process consisting of a iodinated(I-5) resin, a one-micron filter and an absorbtion system used for pH corrections and removal of iodine residual that could affect taste. The straws were evaluated for the removal of Klebsiella terragena bacteria in general non-challenging and challenging test waters at pH 5, 7 and 9 and at 5°C and 25°C, according to EPA guidelines. Replicate 100-ml samples from the straw were collected, quenched to remove residual iodine and then filtered through 0.45 micron filters. Filters were cultured on plate count agar and a repair medium consisting of trypticase soy agar incubated at 35°C for two hours, then overlaid with violet bile agar and reincubated for 48 hours. Greater than six log reductions were achieved in both test waters at pH 7 and 9 and at both temperatures as required by EPA guidelines. While very effective against bacteria, the straws must also be evaluated for the removal of protozoan cysts and enteric viruses beore a military application is determined.					
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PREFACE

This study was performed in support of the U.S. Army Tank-Automotive & Armaments Command (TACOM), Warren, MI 48397-5000 which provided funds and the water purification straws to be tested. It was conducted at the U. S. Army Soldier Systems Center, Natick, MA, from October, 1999 to July, 2000, under Program Element Number 654713, and Work Unit Number JO9CP12W.

The Tank-Automotive & Armaments Command (TACOM), which has the mission for water purification devices, initiated the study of personal water purification straws, with the support of the Soldier Enhancement Program, in response to an Operational Requirements Document (ORD) written by the U.S. Army Combined Arms Support Command and the Fort Lee Directorate of Combat Developments for Quartermaster, Fort Lee, VA. The ORD tasked the Army to provide the individual soldier with a non-developmental, commercial, off-the-shelf, improved state-of-the-art, non-pumping system for emergency treatment of field water, with superior performance enhancements to both filtration and improvements to palatability. This research report presents the bactericidal validation testing of such a system incorporated into a personal water purification straw manufactured by Tech Supply of Virginia (TSV), LLC, Chesapeake, VA.

The use of trade names in this report does not constitute an official endorsement or approval of the use of any commercial product. This report may not be cited for purpose of advertisement.

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BACTERICIDAL EFFICACY OF A PERSONAL WATER PURIFICATION STRAW

INTRODUCTION

The American soldier uses iodine tablets (1, 2) and Chlor-Floc tablets (3) for emergency treatment of field water supplies. Because of certain deficiencies with the tablets such as poor palatability and lengthy disinfection, especially for protozoan cysts at low temperatures (3), a superior system was needed for safe point of use water purification as an alternative or replacement for the tablets.

A recent Operational Requirements Document (4) tasked the Army to provide the individual soldier with an improved state-of-the-art, non-pumping system for emergency treatment of field water, with superior performance enhancements to both filtration and improvements to palatability. The capabilities required to produce potable water must be in accordance with standards set by the National Sanitation Foundation International (5), the U. S. Environmental Protection Agency (6), and the U. S. Army Drinking Water Standards (7) and are as follows: a) provide sufficient disinfection to inactivate viruses and bacteria; b) provide sufficient filtration to remove protozoan cysts; c) provide sufficient water for one soldier in a temperate environment for two weeks (6 quarts a day); d) be compatible with existing canteens and provide sufficient flow to comfortably drink treated water; and e) reduce turbidity of raw water and improve palatability over existing tablets.

The TSV straw system (Tech Supply of VA, LLC, Chesapeake, VA. 23320) had the potential to satisfy Army requirements and consisted of proven multistage processes (8, 9). The iodinated (I_5) resin, a demand release resin, disinfects with a single pass by creating an electrostatic charge that releases an appropriate amount of iodine to kill waterborne organisms that come in contact with it. A one-micron filter removes pathogenic cysts such as *Giardia* and *Cryptosporidium*. The purification process reportedly takes place as the water passes through the straw so that the soldier can hydrate on the move. If effective, there will be no waiting for disinfection, little or no exposure at the water source, no pumping, no tablets and no iodine taste. Storage of water may also be unnecessary which will lighten the soldiers load.

Improved palatability and water quality will increase water consumption and ensure that dehydration does not occur. The personal water purification straw will be used in the same operational concept as the existing water purification tablets and will not impact on current Army Support Systems, or Force Structure. The following study was conducted to validate the efficacy of the TSV personal water purification straw and determine its suitability for the military.

MATERIALS AND METHODS

Media and Buffered water

- (i) Plate Count Agar (PCA).
- (ii) Trypticase Soy Agar (TSA).
- (iii) Trypticase Soy Broth (TSB)
- (iv) Violet Red Bile Agar (VRBA).
- (v) Butterfields phosphate sterile buffered water (SBW)

All media were from Difco, Detroit, Mich.

Bacterial Repair Medium

To recover injured as well as non-injured *K. terrigena* in water recovered from the straw, a repair medium was used (10, 11). This consisted of pre-poured TSA, on which the filter was placed, incubated at 35°C for 2 hours and then overlaid with an equal volume of VRBA.

Test Waters

Testing in EPA test water #1 represented a normal, non-challenging phase of testing and was tested at 25°C and pH 7.0. Testing in EPA #2 test water represented a challenging phase of testing for halogen disinfectants and was always tested in cold water at 5°C and at pH 5.0 and pH 9.0. All test waters were made up in one-liter volumes and sterilized by autoclaving (6).

Personal Water Purification Straws

Straws (Tech Supply of VA, LLC, 1302 Sage Ct., Chesapeake, VA) incorporate a multistage process consisting of a one-micron filter followed by a column of iodinated (I₅) resin followed by an absorption / scavenger system that corrects for pH and removes residual iodine. The straw is 21.7 cm (8.5 in) long x 2.3 cm (7/8 in) in diameter and weighs 56.7 g (2oz).

Bacterial Challenge of Straws

(i) **Bacteria.** The bacteria used were *Klebsiella terrigena*, ATCC 33257 (3, 6). *K. terrigena* was transferred two or three times in TSB (Difco, Detroit, MI) and then spread on PCA to form a lawn of growth. Incubation was at 35°C for 24 hours.

(ii) **Inoculum.** Cells of *K. terrigena* were washed off the PCA with 5 ml of SBW and vortexed for two minutes. The inoculum was adjusted turbidimetrically in a Ratio/XR turbidimeter (Hach Co., Loveland, Colo.) to deliver 10⁸ colony forming units (CFU) per ml. Turbidity was measured as nephelometer turbidity units (NTU).

(iii) **Challenge.** One liter of EPA test water was inoculated to achieve 10⁸ CFU's per liter. Plate counts on PCA were performed each time to confirm the cell numbers. The temperature of the water was equilibrated at 5 and 25°C. Three of the straws were tested consecutively in all three of the test waters and were tested again after flowing several quarts of distilled water and lake water through them. Three straws were also tested in each EPA test water.

Bacteriological Testing Procedure

- (i) Rehydrate straw in sterile deionized water for 5-10 minutes.
- (ii) Inoculate one-liter test water with 10⁸ *K. terrigena* /L.
- (iii) Fill glass column (25mm, ID) to achieve 2-foot head above straw.
- (iv) Gravity flow rate through the straw was 50 ml/minute).
- (v) Discard first 100 ml through the straw. Measure pH and iodine residual.
- (vi) Aseptically collect three 100 ml volumes (second, fourth and sixth) for culturing.

Use volumes collected in between for pH and iodine measurements.

(vii) Quench residual iodine by adding 0.1ml of sterile 10 % sodium thiosulfate solution per 100 ml of treated water.

(viii) Filter 100 ml of quenched treated water through 0.45-micron filter and place filter on pre-poured PCA or TSA agar plates. After two hours at 35°C, overlay TSA with an equal volume (10ml) of VRBA.

(ix) Incubate all plates at 35°C for 48 hours.

CONTROLS

(i). Numbers controls consisted of plate counts from the inoculated and untreated test waters on PCA, from which the log reduction was calculated.

(ii). Recovery controls consisted of 100 ml of water which was flowed through the straw, quenched, re-inoculated with 10^7 of *K. terrigena*, diluted and counted on PCA.

(iii). Filter controls to determine percent recovery and appearance of *K. terrigena* on the filter, consisted of appropriate dilutions of the recovery control calculated to deliver 30 to 50 CFU's when 100 ml was passed through a 0.45 micron filter, which was then cultured on the repair medium in the same manner as the test samples.

(iv). Agar sterility controls consisted of plated medium.

RESULTS AND DISCUSSION

Straws tested in all three test waters. Table 1 shows the average log reduction (3 replications) for three of the straws, each tested consecutively in each of the three EPA test waters (6). Tests were conducted at two pH values because although iodine is bactericidal over the entire pH range, it is more cysticidal and virucidal at pH 5.0 than at pH 9.0 (2, 3, 12). The low temperature (5°C) of EPA #2 water served to slow the germicidal activity of iodine. The straws remained effective and reduced the *K. terrigena* challenging bacteria by more than six log units in EPA #1 water and in cold EPA #2 water at pH 9.0 as required by EPA guidelines (6). However, at pH 5.0 in cold EPA #2 test water, only 5 to 5.5 log reductions were achieved.

TABLE 1. Bactericidal efficacy of personal water purification straws tested consecutively in three EPA test waters

Test water	Temp	pH	Rep.	Average log reduction ^a		
				Straw No.		
				8	11	14
EPA # 1	25 C	7	3	>6	>6	>6
EPA # 2	5 C	5	3	5.2	5.0	5
EPA # 2	5 C	9	3	>6	>6	>6

^a*Klebsiella terrigena*, 10^8 per liter

Heterotrophic plate count. A heterotrophic plate count on PCA was encountered during testing of these three straws as shown in Table 2. Since the challenge waters were sterile and contained only *K. terrigena* inoculated into it, the HPC bacteria were indigenous to the straw and may have been present and multiplied in the scavenger/absorption material in the top section of the straw (Figure 1) during wet storage, a practice that is not recommended. These bacteria were not exposed to the I₅ resin, which was in the lower chamber of the straw. They were typical, harmless environmental contaminants consisting of gram positive cocci from yellow pigmented colonies, gram negative, motile, rod shaped bacteria from orange and yellow colonies, gram negative, oxidase positive, rod shaped bacteria from off white colonies, and gram positive sporeforming bacilli. To reduce the HPC on plates and avoid interference with counts of the challenge organism, even though *K. terrigena* was distinctive and easily distinguishable, the effluent from straws was thereafter plated on TSA overlaid with VRBA (10, 11). After repairing their injury on TSA, the VRBA selected and differentiated *K. terrigena*, a coliform, and inhibited the growth of the environmental contaminants. The effectiveness of this procedure is shown by the lower counts in the third column with EPA #2 water at pH 9.

TABLE 2. Heterotrophic plate count of effluent from new personal water purification straws tested consecutively in three EPA test waters

Straw	CFU per 100 ml of test waters		
	P C A		VRBA/TSA
	EPA #1, pH 7 25° C	EPA #2, pH 5 5° C	EPA #2, pH 9 5° C
08	272	290	200
08	305	1350	200
08	211	4000	58
11	>250	3150	8
11	>250	3270	19
11	>250	3000	5
14	>250	>3000	201
14	>250	>3000	7
14	250	>3000	7

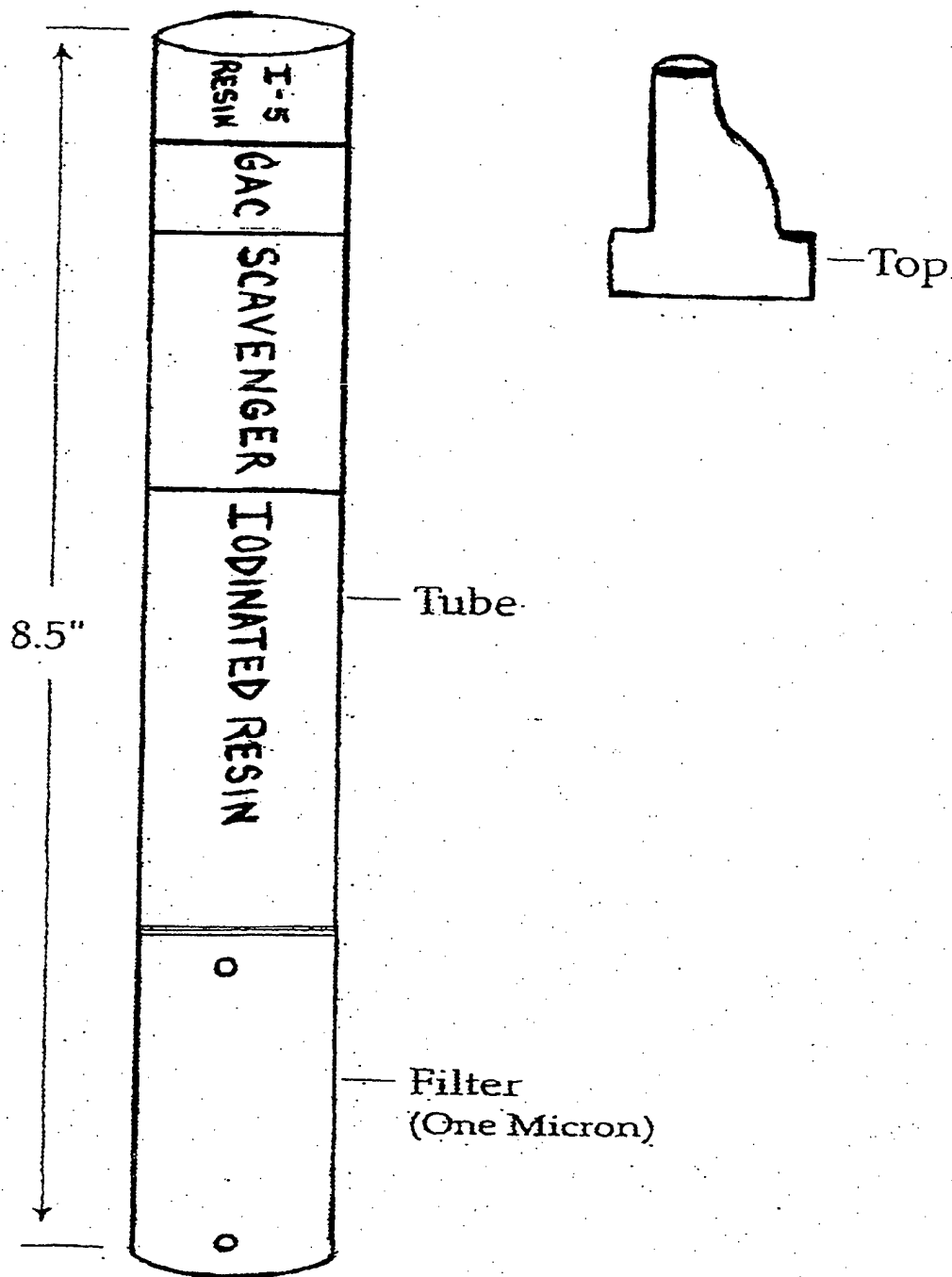


FIG 1. Personal water purification straw. Reproduced with permission of Tech Supply of Virginia, LLC.

New Unused Straws. Table 3 shows the bactericidal efficacy of unused straws tested in triplicate in each of the three EPA test waters (6). All straws tested complied with EPA guidelines by achieving greater than a six log reduction of *K. terrigena* in the respective test waters. To recover injured, as well as non-injured bacteria from the effluent collected from the straws, TSA overlaid with VRBA repair medium was used (10, 11). No HPC was encountered with these straws because they were not previously used or stored wet before testing. Therefore, if environmental contaminants were present they were less than 10/ml and had no time to multiply.

TABLE 3. Bactericidal efficacy of new unused straws in each of three EPA test waters

Test Water	pH	Temp. °C	Flow Rate per minute	Log Reduction in Straw								
				1	2	3	4	5	6	7	8	9
EPA 1	7	25	50 ml	>6	>6	>6						
EPA 2	5	5	50 ml				>6	>6	>6			
EPA 2	9	5	50 ml							>6	>6	>6

Straws. Table 4 shows the continued bactericidal efficacy of these straws after considerable usage and tested under worst case conditions in cold EPA # 2 test waters. Several quarts of distilled water (DW), followed by lake water (LW), were passed through the same three previously tested straws and tested again for bacterial removal. Straw #8 was challenged with 17 liters DW, followed by 9 liters of LW (NTU 11 to 17); straw # 11 was challenged with 22 liters of DW followed by 7 liters of LW (NTU 8.7); and straw #14 was challenged with 17 liters of DW followed by 16 liters of LW (NTU 2.8 to 3.8). Challenge with LW was discontinued because of reduced gravitational flow rates, shown in Table 5. All three straws effectively reduced *K. terrigena* by more than six log cycles with the exception of straw #14 which was probably defective when tested at pH 5.0. It was effective when tested earlier at pH 9.0. A HPC was encountered as before, but was reduced by overlaying TSA repair medium with VRBA.

TABLE 4. Bactericidal efficacy of personal water purification straws after challenging with distilled and lake waters ^a.

Test water	Temp. °C	Ave. pH Water after straw	Reps	Average Log Reduction ^b by straw no.		
				8	11	14
EPA #2, pH 9	5	6.3	3	>6	>6	>6
EPA #2, pH 5	5	6.2	3	>6	>6	<1

^a 32-48 Quarts Distilled and Lake Waters

^b *Klebsiella terrigena*, 10⁸ per liter

Flow Rates. Table 5 shows the average gravitational flow rates during testing of the three straws challenged with distilled and lake waters and the total volume of water passed through each straw. The flow rates were considerably reduced during collection of the 100 ml samples compared to the initial flow rates which were two minutes per 100 ml sample. Since three inches of vacuum are applied by the human mouth (13), water could still, undoubtedly, be drawn through the straws for drinking.

TABLE 5. Average gravitational flow rates of personal water purification straws during testing after challenging with distilled and lake waters.

Straw	EPA #2, pH 5 min/100 ml	EPA #2, pH 9 min/100 ml	Challenge water Quarts
08	10.6	7.3	32
11	9.6	4.3	34
14	3.0	3.2	48

Controls. Plate counts from all controls verified the bacterial challenge and recovery from the straw and were as estimated by a NTU standard curve. Numbers controls averaged 1.93×10^8 CFU's / liter. Recovery controls averaged 1.9×10^8 CFU's / liter. Percent recoveries of *K. terrigena* on the filter controls ranged from 95% to 125% in both test waters.

CONCLUSIONS

The personal water purification straws tested were effective and will remove 99.9999% of the waterborne bacteria as required by EPA (6). However, the straws must be challenged by enteroviruses and protozoan cysts before they can be recommended for use by the soldier in the field. The straw will save valuable time in procuring water at the water source and allow more time for the mission or escape and evasion. The soldier can fill the canteen with water and purify it while on the move by sipping on the straw whenever hydration is needed. As water is drawn through the straw, the purification process takes place. The straw will filter more than 12 gallons of water. Since the straw only weighs two ounces it can easily be integrated on the soldier and may lighten his load in the field by making it unnecessary to carry or store purified water.

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